## PS 2347 ADVERSE EFFECT OF ALUMINIUM ON SECRETARY PRODUCTS AND ANTIOXIDANT ENZYMES IN THE EPIDIDYMIS OF RATS—PROTECTIVE EFFECT OF VITAMIN E.

R. Venugopal[1], S. Onorine Marcelline[1], P. Subbarayalu[2] and S. Viswanadhapalli[3]. *[1]Zoology, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry 605 008, Puducherry, India, [2]Greehey Children Cancer Research Institute, University of Texas Health Science Centre at San Antonio, San Antonio, TX and [3]Medicine/Nephrology, University of Texas Health Science Centre at San Antonio, San Antonio, TX.*

Aluminium has several industrial uses and is widely used in the production of medicines like analgesics, antacids and anti-diarrheal besides finding use as a food additive and water purification agent. Considering the toxic nature of aluminium with less work on reproductive system, the present study was planned to investigate the effect of aluminium chloride on secretary products, enzymatic and non-enzymatic antioxidants and its possible recovery by vitamin E treatment in the epididymis of adult rats. Adult male rats were administered with aluminium chloride, 100 mg/kg body weight, orally, daily for 45 days. Second group of rats were treated with aluminium chloride along with vitamin E. Third group of rats treated with vitamin E alone and the fourth group served as withdrawal group. All the groups of rats were compared with the control group. At the end of the experimental period the animals were sacrificed and the epididymis was dissected out. Antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase were markedly diminished in the epididymis of aluminium chloride treated animals. The non-enzymatic antioxidants vitamin C and vitamin E were also decreased. The epididymal lipid peroxidation and hydrogen peroxide were significantly increased. Glycerine phosphoryl choline, sialic acid, carnitine and acetyl carnitine contents were markedly decreased. Vitamin E treatment counteracted the effect of aluminium chloride. In the withdrawal group most of the parameters were brought back to near normalcy. The present study suggests the reproductive toxicity of aluminium by altering secretary products and inducing the oxidative stress in the epididymis and its possible recovery by vitamin E treatment.

## PS 2348 APOPTOSIS INDUCED BY 4-TERT-OCTYLPHENOL IN RAT TESTIS AND THE INTRINSIC PATHWAY ACTIVATION.

Q. Bian and H. Wang. *Jiangsu Center of Disease Control and Prevention, Nanjing, China.*

Concerns regarding 4-tert-octylphenol (OP) have increased as this chemical displays estrogenic activity. The objective of this study is to elucidate the molecular mechanism(s) underlying the detrimental effects associated with OP as well as to explore whether intrinsic pathway of apoptosis induced by OP was activated. According to our previous in vivo study, the expression levels of some apoptotic related genes, i.e. Bcl-x and death effector domain-containing protein (spermatogenesis, apoptosis regulator activity) and sperm membrane protein (YWK-II) were decreased in adult rats treated by OP. Here, the Bcl-2 family proteins expression and intrinsic pathway activated signals were further observed by western blot and flow cytometric analysis (FCM).
The results showed increased apoptosis of testis cells occurred in a concentration-dependent manner by FCM assay (Fig 1). The expression of Bcl-xL was down-regulated, while the expression of active Bax up-regulated (Fig 2). OP also down-regulated the expression of 32 kDa procaspase-3, which was cleaved to generate active subunit (17 kDa) and could induce Cyt C release (Fig 2 & 3).
Taken together, these results suggest that OP may induce rat testis apoptosis and could trigger apoptosis via mitochondria-dependant intrinsic pathway by regulation of Bcl-xL/Bax, Cyt C release and caspase-3 activation. Moreover, these findings may provide useful indicators of the adverse effects of OP on male reproductive system and prove particularly important in elucidating that Bcl-2 family proteins may play a key role in testicular function change elicited by OP.

## PS 2349 DECIPHERING MECHANISMS UNDERLYING PROLONGED MALE INFERTILITY FOLLOWING A CLINICALLY-RELEVANT MULTICYCLE CISPLATIN TREATMENT.

J. Harman and J. Richburg. *University of Texas Austin, Austin, TX.*

A typical clinical regimen consists of repeated cycles of 5-7 daily injections of low dose cisplatin with a 1-2 week recovery period. An unfortunate side effect of cisplatin exposure in males is a prolonged, sometimes permanent, infertility. Previously, we developed a clinically-relevant treatment paradigm in adult C57 mice (repeated cycles of 2.5mg/kg/day for 5 d followed by a 16 d recovery period) and discovered that the severity of testicular damage is more dependent on the number of cycles of treatment than the cumulative dose. Theoretically, spermatogonial stem cells (SSCs) should be able to repopulate the testis after cisplatin exposure has ceased. We hypothesize that an increase in the mitotic activity of SSCs during the initial exposure to cisplatin renders them increasingly susceptible to cisplatin-induced injury during the next treatment cycle, underlying the mechanism of treatment-induced infertility. Here we investigate Sertoli cell (SC) factor(s) that stimulate SSCs to enter the cell cycle after cisplatin exposure; namely, glial cell line-derived neurotrophic factor (GDNF). Adult C57 mice were exposed to 5 daily intraperitoneal injections of 2.5 mg/kg cisplatin. Mice were sacrificed at days 1, 3, 7, and 16 of the recovery period and testes removed for immunohistochemical (IHC) analysis. In order to measure mitotic activity, BrdU was administered intraperitoneally 1.5 hours prior to sacrifice. IHC was performed using antibodies against GDNF, incorporated BrdU, and PLZF (undifferentiated spermatogonia marker). IHC analysis of BrdU showed a 1.37-fold increase in the proliferative rate of early germ cells over controls. GDNF immunostaining in cisplatin-treated mice was particularly prominent along the basal membrane, the region where SSCs reside. PLZF labeling decreased immediately following cessation of treatment, increased by day 7 and returned to control levels by day 16. Future experiments are targeted to test the direct role of GDNF, as well as other potential regulatory factors, in increasing the sensitivity of SSCs to cisplatin-induced injury.

## PS 2350 ZINC DEFICIENCY EXACERBATES DIABETIC TESTICULAR CELL DEATH BY DOWN-REGULATION OF AKT EXPRESSION AND FUNCTION: ESSENTIAL ROLES OF PTEN, PTP1B, AND TRB3.

Y. Zhao[1, 2], J. Dai[2], Y. Tan[2], G. Wang[1], L. Cai[2, 1] and W. Li[1]. *[1]Cancer Center, First Bethune Hospital of Jilin University, Changchun, Jilin Province, China and [2]KCHRI at the Department of Pediatrics, University of Louisville, Louisville, KY.*

Since zinc plays an important role in the spermatogenesis and diabetic patients are often with zinc deficiency, this study was to investigate the impact of zinc deficiency to diabetic effects on testicular cell death and possible mechanisms. Type 1 diabetic model was induced in FVB mice with multiple low dose of streptozotocin. Zinc deficiency was induced by zinc chelator, N, N, N', N'-tetrakis (2-pyridylemethyl) ethylenediamine (TPEN). After diabetes onset, both hyperglycemic and age-matched control mice were given TPEN intraperitoneally for four months. Testicular zinc levels were decreased in diabetes and TPEN groups, and further decreased in diabetes/TPEN group. Testicular cell apoptosis was increased in diabetes group and further increased in diabetes/TPEN group. For mechanistic study, Western blot assay revealed that Akt-mediated glucose metabolism signaling was down-regulated in the testis of diabetes group and further decreased in diabetes/TPEN group, reflected by reduced phosphorylation of both Akt and GSK-3β and increased phosphorylation of GS along with the disarrangement of fatty acid metabolism (increased expression of PPAR-α, decreased expression of Sirt1 and PGC-1α, decreased AMPK phosphorylation). Furthermore, Akt negative regulators PTEN, PTP1B and TRB3 all increased in diabetic testis and further increased in the testis of diabetes/TPEN. These studies suggest that zinc deficiency significantly exacerbated diabetic induction of testicular cell death probably via down-regulation of Akt expression and function by up-regulation of Akt negative regulators. Therefore, prevention of zinc deficiency for diabetic patients is of vital importance in order to avoid the exacerbation of diabetic inhibition of glucose metabolism in the testis.

## PS 2351 EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS RELATED TO SPERM QUALITY AND DNA INTEGRITY.

A. Jeng[1], C. Pan[2] and M. Wu[3]. *[1]Old Dominion University, Norfolk, VA, [2]Institute of Occupational Safety and Health, Taipei, Taiwan and [3]Kaohsiung Medical University, Kaohsiung, Taiwan.* Sponsor: Y. Huang.

The objectives of this study were to explore i) whether exposure to polycyclic aromatic hydrocarbons (PAHs) contribute to the alteration of male sperm quality and DNA integrity of coke-oven workers, and ii) whether gene polymorphism can modulate the effect induced by PAHs . Our specific aims were to i) determine the correlation between 16 individual PAH species and their biomarkers and sperm quality and DNA integrity; ii) determine sperm oxidative damage by identifying specific DNA lesions, iii) assess the correlation between gene polymorphisms and sperm quality and DNA integrity. A longitudinal study included repeated measurements to account for PAH exposure as it relates to toxic effects on sperms during

spermatogenesis. Personal breathing-zone air samples and urine samples were collected to determine PAH external and internal exposure levels, respectively. Semen was collected to assess sperm quality, DNA adducts, and oxidative damage. Blood samples were collected for genotyping. The high exposed group had a significantly lower percentage of motile, viable and normal morphological sperms as compared to the control. The PAH exposed group experienced higher DNA fragmentation percentages, 8-oxodG concentrations, bulky DNA adducts, and BaP like DNA adducts. The concentration of 1-OHP levels negatively correlated with normal sperm morphology and motility. PAHs with higher molecular weights tend to correlate with sperm quality and DNA fragmentation. GSTM1 null and CYP1A1 Msp1 men had higher sperm DNA fragmentation and 8-oxodG concentration. Exposure to PAHs altered sperm quality and increased oxidative DNA damage. Genetic polymorphisms influence the susceptibility of men to sperm DNA damage related to exposure to PAHs. Research results laid a foundation for future study related to the effects of PAHs on reproductive health and examine gene and environment interaction.

## PS 2352 EVALUATION OF REPRODUCTIVE FUNCTION IN MALE CYNOMOLGUS MONKEYS—TESTICULAR VOLUME AND SPERM ANALYSIS PARAMETERS.

K. Mizuyoshi, S. Sakaki, K. Motokawa, R. Oohara, S. Sakoda, K. Watanabe, Y. Taniguchi, J. Nakano, Y. Harada, T. Sugimoto, Y. Ooshima and R. Nagata. *Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories, Ltd., Kagoshima-shi, Kagoshima-ken, Japan.*

Nonhuman primates (NHPs) are frequently used for toxicity studies of biopharmaceuticals since they are often the only experimental animals to express pharmacologic responses similarly to humans. There is an associated need to evaluate NHP male fertility abilities. We investigated the correlations between testicular volume, and sperm analysis and serum testosterone parameters in untreated males. One hundred and eighty-four animals aged 57 to 98 months and weighing 4.2 to 8.7 kg were used. Testicular volume was calculated from the major and minor testicular axes. Semen was collected by electroejaculation, and examined to determine sperm count and motility (%motile sperm, curvilinear velocity, total lateral head displacement amplitude, head osculation frequency, linearity, track velocity, straight line velocity, and track linearity; IVOS sperm analyzer) and the rate of morphological malformation. Blood was collected twice for serum testosterone measurement by ELISA. We also investigated the effects of (±)-3-chloro-1,2-propanediol (α-chlorohydrin) on sperm motility *in vitro*. A slightly positive correlation between sperm count and age was noted. Testicular volume was positively correlated with sperm count and serum testosterone level. %motile sperm was negatively correlated with malformation rate. No correlations were noted between serum testosterone concentration and sperm count, or sperm motility and malformation rate.
α-chlorohydrin affected sperm velocity *in vitro* at a concentration of 1% with 5-minute incubation.We concluded that testicular volume and sperm analysis parameters are good indicators for selection of cynomolgus monkeys in toxicity studies.

## PS 2353 UNDERNUTRITION INHIBITS FETAL RAT TESTIS STEROIDOGENESIS.

K. Johnson, E. McDowell and J. Pike. *Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Wilmington, DE.*

Humans born small for gestational age are at increased risk of male reproductive malformations, including cryptorchidism and hypospadias. The mechanistic basis for this increased risk is unknown. Because both hypospadias and testis maldescent can be caused by reduced in utero androgen action, we hypothesized that intrauterine growth restriction is associated with an inhibition of fetal testis testosterone production. To test this hypothesis, we used a Wistar rat macronutrient food restriction model from gestational day (GD) 3 through GD17. Two global food restriction levels were examined: 50% and 30% of an ad libitum diet. On GD17, fetal testis steroidogenic mRNA levels and ex vivo testosterone production were measured. Both undernutrition models significantly reduced maternal body weight, and intrauterine growth restriction in both models was confirmed by a significant decrease in GD17 pup weight. Litter size was unaffected. Both groups showed significant reductions in GD17 testis mRNA for genes in the steroidogenic pathway (Cyp11a1, Cyp17a1, and Star) and the cholesterol import pathway (Scarb1). The inhibition of steroidogenic gene expression was accompanied by a significant decrease (40%) in GD17 testis testosterone production. No significant difference in any steroidogenic endpoint was detected between the two undernutrition models. Based upon these data, we conclude that rat maternal undernutrition leads to fetal intrauterine growth restriction and inhibits fetal testis testosterone production. Because this endocrine disruption occurs during the fetal masculinization programming window, boys born small for gestational age may be at increased risk of cryptorchidism and hypospadias because of reduced androgen production during a critical developmental window.

## PS 2354 RESVERATROL RESTORES FERTILITY INDICES IN MALE RATS ORALLY ADMINISTERED WITH BENZO(A)PYRENE.

A. E. Archibong[1], M. S. Niaz[2] and A. Ramesh[2]. *[1]Physiology, Meharry Medical College, Nashville, TN and [2]Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN.*

Benzo(a)pyrene (BaP) is a semi-volatile, persistent environmental pollutant. Apart from inhalation of BaP through cigarette smoke and occupational settings, humans are also exposed to BaP through consumption of contaminated foods. The objective of this study was to assess the ability of resveratrol (RVT) to block the effect of orally administered BaP on male fertility indices. Adult male F-344 rats were randomly assigned to receive BaP only (5 mg/kg) or RVT (50 mg/kg) + BaP or vehicle (tricaprilyn; VEH control) for 60 days. Thereafter, all animals were anesthetized to facilitate blood samples collection for serum testosterone measurement. Subsequently, testes and epididymides were harvested, weighed and stored spermatozoa recovered for the determination of progressive motility and sperm density. The right testes of rats in each group were subjected to H&E staining post fixation in buffered formalin. Testis weights did not differ among BaP-treated, RVT + BaP and VEH control rats. However, mean epididymal weight was reduced among BaP-treated rats, an effect that was blocked by RVT compared to VEH control treatment (BaP, 0.44 ± 0.01; RVT + BaP, 0.50 ± 0.01; VEH, 0.48 ± 0.01 [P<0.01]). Similarly, RVT blocked the ability of BaP to reduce both stored sperm motility (RVT + BaP, 78.0 ± 3.3; BaP, 57.0 ± 4.02; 82.0 ± 2.1 [P<0.005]) and density (RVT + BaP, 84.0 ± 4.0; BaP, 36.0 ± 6.0; VEH, 82.0 ± 4.0 [P<0.05]). H&E staining showed a significant disruption in the integrity of the Leydig cell compartment of the testes of BaP-treated versus those of control rats, a condition that was blocked by RVT. Interestingly, serum testosterone concentrations were significantly reduced in BaP-treated versus control rats. However, testosterone concentrations were not affected when BaP was co-administered with RVT. These data suggest that RVT blocked BaP-induced disruption in endocrine-regulated fertility indices in male rats by maintaining the secretion of testosterone (funded by NIH grants 1S11ES014156-05 and 1R01CA142845-01A1).

## PS 2355 TESTICULAR AND EPIDIDYMAL HISTOLOGIC CHANGES AROUND THE PERIOD OF SEXUAL MATURATION IN YUCATAN BOARS.

T. Evans[1], J. Trickey[2], L. D. Brown[2] and G. Bouchard[2]. *[1]Veterinary Medical Diagnostic Laboratory University of Missouri, Columbia, MO and [2]Sinclair BioResources LLC, Auxvasse, MO.*

Preclinical guidelines often specify the use of prepubertal, pubertal, or sexually mature animals. However, "puberty" and "sexual maturity" can be defined in a number of different ways which reflect androgen production, including the onset of mounting behavior, penile erection and/or ejaculation ± sperm capabilities, or, histologically, by a "threshold" portion of seminiferous tubules engaged in spermatogenesis ± epididymal sperm. Furthermore, "sexual maturity" can also be interpreted slightly differently, depending on the type of toxicology study programs (e.g., DART versus repeat dosing studies). Since Yucatan boars have been reported to reach "puberty" as early as 12 weeks or as late as 16 to 20 weeks of age, it is critical, regardless of how the stages of sexual development are defined, to know what is happening histologically in the testes at these various ages. Modified Davidson's-fixed and PAS-stained testicular and epididymal sections were evaluated from 12-, 14-, 16-, 18-, 20-, 22-, and 24-week-old Yucatan boars (n=minimum of 4/age group). Approximately 200 seminiferous tubules were evaluated per testis for immature/mature tubules. The proportion of the total number of seminiferous tubules represented by "mature tubules" was calculated. The presence of sperm in the caudae epididymides was also noted. Round spermatids began to appear at 12 weeks of age, and a majority of 14-week-old boars had seminiferous tubules containing both round and elongate spermatids. While sparse numbers of sperm appeared in the epididymides of one boar at 14 weeks of age, at least half of the 16- and 18-week-old boars exhibited some spermiation with sperm in the excurrent duct system. By 20 weeks of age almost all seminiferous tubules were "mature", with sperm present in the epididymides. These novel data can be taken into consideration, along with other indices of sexual development, when designing toxicology experiments of varying durations which require Yucatan boars at a given stage of sexual maturity.

# The Toxicologist

Supplement to *Toxicological Sciences*

## 51st Annual Meeting and ToxExpo™

**March 11–15, 2012 • San Francisco, California**

SOT 2012 San Francisco California

An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

www.toxicology.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 126, Issue 1
March 2012

www.toxsci.oxfordjournals.org